PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	27 NOV 2006
Applicant's or agent's file reference STAN-337WO	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US04/38830	18 November 2004 (18.11.2004)	20 November 2003 (20.11.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC: C12P 21/06(2006.01)
USPC: 435/69.1

Applicant

The Board of Trustees of the Leland Stanford Junior University

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	16 October 2006 (16.10.2006)	Thane Underdahl Telephone No. (571) 272-1600

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/38830

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/38830

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims NONE	YES
	Claims 1-18	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-18	NO
Industrial applicability (IA)	Claims 1-18	YES
	Claims NONE	NO

2. Citations and explanations:

Claims 1-13, 16, and 18 lack novelty under PCT Article 33(2) as being anticipated by Swartz et al. (U.S. Patent 6,337,191, published January 8, 2002).

These claims are drawn to the *in vitro* synthesis of biological macromolecules using a reaction mix comprising a phosphate free energy source in the presence of exogenous phosphate. The phosphate free energy source can be glucose, glutamante or pyruvate. The exogenous phosphate concentration is between 1mM and 20mM of either potassium, magnesium or ammonium phosphate. The phosphate source is released during the reaction. The synthesis of the biological macromolecules is via translation from mRNA from a DNA template. The reaction mix also comprises magnesium at a concentration of 5mM to 20mM. The reaction mix also comprises one or more of spermine, spermidine, and putrescine, as well as nucleoside monophosphates. Once the protein synthesis is started no exogenous nucleotide triphosphates are added.

Swartz et al. teach a method of *in-vitro* synthesis of proteins using phosphate free energy sources (col 2, lines 53-63) such as glucose and pyruvate (col 3, lines 50-57) the media also contains non-catalytic amounts of glutamate (col 10, line 49). The reaction mix contains 6.7mM of potassium phosphate. The reaction mix also contains nucleotide triphosphates (col 10, line 48) that will release phosphate upon the formation of mRNA from a DNA template (col 8, lines 1-10). The process can be performed in a batch or continuous reaction (col 7, lines 60-65). The reaction mix contains 15mM of magnesium (col 10, line 50) and contains 2% of polyethylene glycol (col 10, line 53). The mix also comprises spermine and spermidine (col 8, line 19) as well as cAMP (col 10, line 49). Once the protein synthesis reaction was started no exogenous nucleoptide triphoshpates were added to the mix (col 12, lines 66-67).

Therefore the reference shows claims 1-13, 16, and 18 lack novelty.

Claims 1-7, and 12-17 lack novelty under PCT Article 33(2) as being anticipated by Swartz et al. (U.S. Patent Application 10/091,538 , published Nov 4, 2002) as supported by Zubay (Annual Review of Genetics, 1973).

These claims are drawn to the *in vitro* synthesis of biological macromolecules using a reaction mix comprising a phosphate free energy source in the presence of exogenous phosphate. The phosphate free energy source can be glutamate, glucose or pyruvate. The phosphate source is released during the reaction. The synthesis can be in a batch or continuous reaction. The reaction mix comprises *E. Coli* extract grown in a glucose and phosphate containing medium. The reaction mix also comprises magnesium at a concentration of 5mM to 20mM. The mix is substantially free of polyethylene glycol.

Chattergee et al. teach a method of *in vitro* synthesis of a peptide/protein or nucleic acid synthesis systems (page 3 paragraph 37). These systems can us non-phosphate containing energy sources such as glucose and pyruvate (page 7, paragraph 76). The reaction mix also contains non-catalyitc amounts of glutamate (page 10, paragraph 104). As the mRNA is synthesized from the nucleic acid template in the system of Chattergee et al. (page 7, paragraph 73) the attached phosphate will be released. The *E.Coli* extract used by Chattergee et al. was made by the method of Zubay (page 10 , paragraph 104). Zubay teach that the E. Coli extract is made from cells grown in buffer containing glucose and phosphate (page 275, Growth of Cells). No polyethylene glycol was added to the reaction mix of Zubay or Chattergee et al.

Therefore the reference shows claims 1-7 and 12-17 lack novelty.

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 1, 2, 4-13, 16, 18 lack inventive step under PCT Article 33(2) over Swartz et al. (U.S. Patent 6,337,191, published January 8, 2002).

These claims are drawn to the *in vitro* synthesis of biological macromolecules using a reaction mix comprising a phosphate free energy source in the presence of exogenous phosphate. The phosphate free energy source can be glucose or pyruvate. The exogenous phosphate concentration is between 1mM and 20mM of either potassium, magnesium or ammonium phosphate. The phosphate source is released during the reaction. The synthesis of the biological macromolecules is via translation from mRNA from a DNA template. The reaction mix also comprises magnesium at a concentration of 5mM to 20mM. The reaction mix also comprises one or more of spermine, spermidine, and putrescine, as well as nucleoside monophosphates. Once the protein synthesis is started no exogenous nucleotide triphosphates are added.

Swartz et al. teach a method of *in-vitro* synthesis of proteins using phosphate free energy sources (col 2, lines 53-63) such as glucose and pyruvate (col 3, lines 50-57). The reaction mix contains 6.7mM of potassium phosphate. The reaction mix also contains nucleotide triphosphates (col 10, line 48) that will release phosphate upon the formation of mRNA from a DNA template (col 8, lines 1-10). The process can be performed in a batch or continuous reaction (col 7, lines 60-65). The reaction mix contains 15mM of magnesium (col 10, line 50) and contains 2% of polyethylene glycol (col 10, line 53). The mix also comprises spermine and spermidine (col 8, line 19) as well as cAMP (col 10, line 49). Once the protein synthesis reaction was started no exogenous nucleoptide triphoshpates were added to the mix (col 12, lines 66-67).

Claim 4 lacks an inventive step under PCT Article 33(3) as being obvious over Swartz et al. (U.S. Patent 6,337,191, published January 8, 2002) as supported by Stryer et al. (Biochemistry, 1995).

This claim is drawn to the use of the glutamate as the phosphate free energy source. Swartz et al. teach that the energy source pyruvate in the presence of glutamate can be converted to alanine via the alanine-glutamate transaminase. However that same reaction (called Alanine aminotransferase by Stryer) produces á-ketoglutarate which could be used in the TCA cycle to produce more energy via NADH (Stryer pages 513 and 630). Stryer also teach that the sum of an aminotransferase and glutamate dehydrogenase produces α-ketoglutarate and generates an additional molecule of NADH (Stryer, page 630). It would therefor be obvious

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for one of ordinary skill in the art to use glutamate as a power source for an in-vitro protein synthesis since two biochemical pathways that use glutamate generate energy via NADH.

Therefore the reference shows the claims 1, 2, 4-13, 16, 18 lack inventive step.

Claims 1, 2, 4, 5-7, and 12-17 lack inventive step under PCT Article 33(2) over Chattergee et al. (U.S. Patent Application 10/091,538 , published Nov 4, 2002) as supported by Zubay (Annual Review of Genetics, 1973).

These claims are drawn to the *in vitro* synthesis of biological macromolecules using a reaction mix comprising a phosphate free energy source in the presence of exogenous phosphate. The phosphate free energy source can be glucose or pyruvate. The phosphate source is released during the reaction. The synthesis can be in a batch or continuous reaction. The reaction mix comprises *E. Coli* extract grown in a glucose and phosphate containing medium. The reaction mix also comprises magnesium at a concentration of 5mM to 20mM. The mix is substantially free of polyethylene glycol.

Chattergee et al. teach a method of *In vitro* synthesis of a peptide/protein or nucleic acid synthesis systems (page 3 paragraph 37). These systems can us non-phosphate containing energy sources such as glucose and pyruvate (page 7, paragraph 76). As the mRNA is synthesized from the nucleic acid template in the system of Chattergee et al. (page 7, paragraph 73) the attached phosphate will be released. The E.Coli extract used by Chattergee et al. was made by the method of Zubay (page 10 , paragraph 104). Zubay teach that the E. Coli extract is made from cells grown in buffer containing glucose and phosphate (page 275, Growth of Cells). No polyethylene glycol was added to the reaction mix of Zubay or Chattergee et al.

Therefore the reference shows the claims 1, 2, 4, 5-7, and 12-17 lack inventive step.